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Lab Resource: Stem Cell Line

Derivation of an aged mouse induced pluripotent stem cell line, IISHDOi005-A

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A B S T R A C T

A mouse iPSC line, IISHDOi005-A, generated from fibroblasts obtained from a mouse C57BL/6J with an age of 1 year and a half, has been obtained. For this purpose, reprogramming factors Oct3/4, Sox2, Klf4, and c-Myc were delivered using Sendai virus.

Resource table.

Unique stem cell line identifier	IISHDOi005-A
Alternative name(s) of stem cell line	CmC57-FiPS4F1
Institution	Instituto de Investigación Sanitaria Hospital 12 de Octubre, i + 12. Madrid, Spain
Contact information of distributor	Dr. M. Esther Gallardo egallardo.imas12@h12o.es
Type of cell line	iPSC
Origin	Mouse
Additional origin info	Age: 1.5 years Sex: Female Ethnicity if known: N/A
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Transgene free (Sendai virus)
Genetic Modification	NO
Type of Modification	N/A
Associated disease	N/A
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	20 September 2017
Cell line repository/bank	N/A
Ethical approval	This study was reviewed and approved by the Institutional Ethical Committee of the "Instituto de Investigaciones Biomédicas Alberto Sols", CSIC-UAM, 406 329 1

Resource utility

The main biological hallmarks of aging include stem cell exhaustion and cellular senescence. Nowadays, there are increasing research efforts to look for anti-aging therapies. The iPSC line reported here could be a very useful resource for modelling aging and for designing therapeutic strategies against it.

Resource details

The mouse iPSC line, IISHDOi005-A, has been generated using a non-integrative methodology that involves the use of Sendai viruses containing the reprogramming factors Oct3/4, Sox2, Klf4, and c-Myc (Takahashi et al., 2007). For this purpose, fibroblasts from a C57BL/6J mouse with an age of 1 year and a half have been obtained from skin. IISHDOi005-A iPSC colonies displayed a typical ES-like colony morphology and growth behaviour (Fig. 1A) and they stained positive for alkaline phosphatase activity (Fig. 1B). The line was confirmed by PCR

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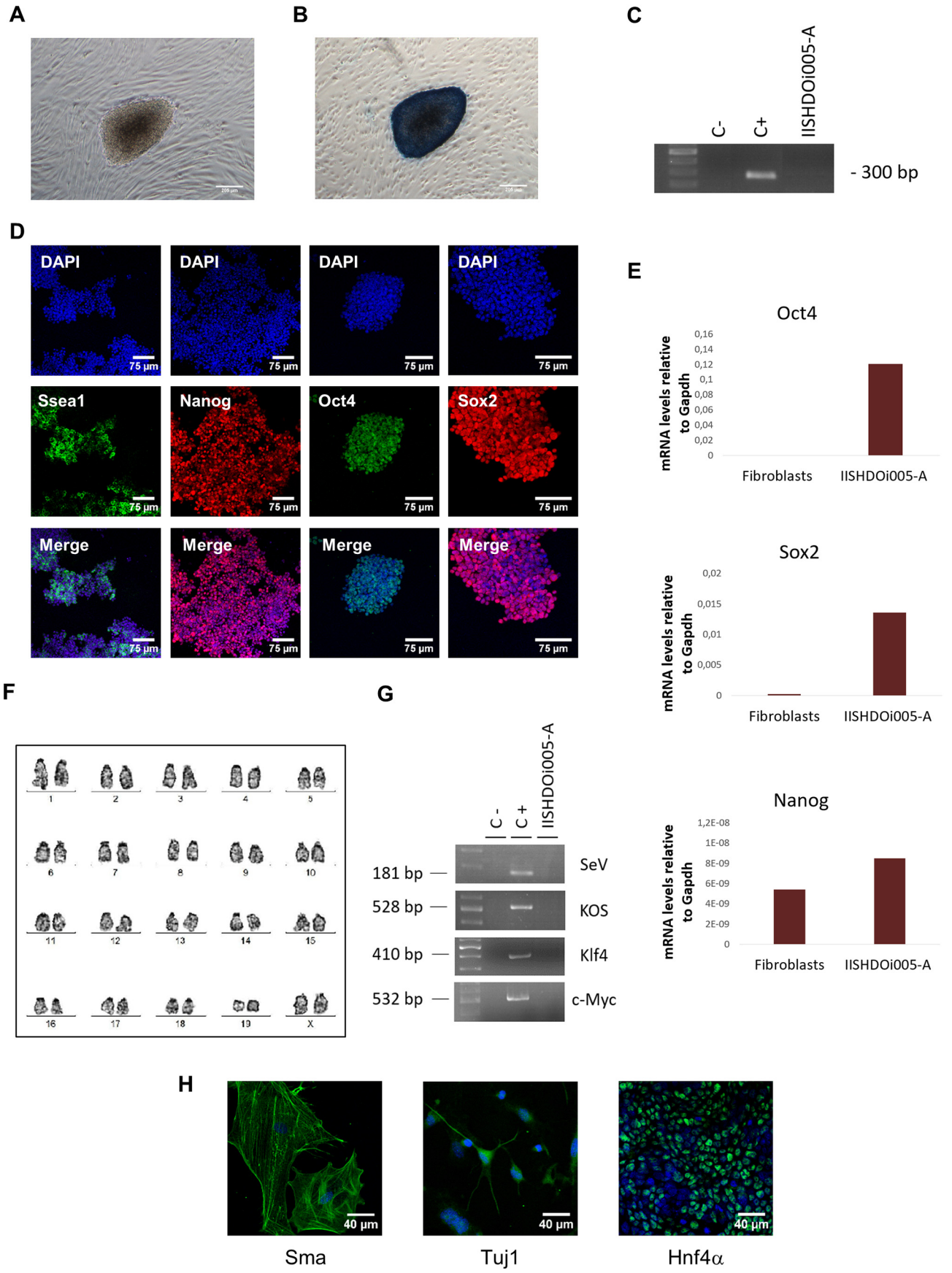

Fig. 1. Molecular and functional characterization of the IISHDOi005-A iPSC line.

analysis to be mycoplasma-negative (Fig. 1C). Immunofluorescence analysis revealed expression of transcription factors Oct4, Nanog and Sox2, and the ES cells surface marker, SSEA1 (Fig. 1D). The endogenous expression of the pluripotency associated transcription factors Oct4, Sox2 and Nanog was evaluated by quantitative real time polymerase chain reaction (qPCR) (Fig. 1E). The iPSC line has been adapted to feeder-free culture conditions and a karyotype analysis after more than 20 culture passages has been performed (Fig. 1F). This analysis displayed a normal (40, XX) karyotype. In addition, we confirmed by DNA fingerprinting analysis that the line IISHDOi005-A was derived from the initial mouse fibroblasts (available with the author). The clearance of the vectors and the exogenous reprogramming factor genes was also confirmed by RT-PCR after twelve culture passages. Primers used allow the specific detection of both human and mouse transgenes because sequences hybridize with both species (Fig. 1G). Finally, the capacity of the IISHDOi005-A iPSC line to differentiate into the three germ layers (endoderm, mesoderm and ectoderm) was analysed *in vitro* using an embryoid body based assay (Fig. 1H).

Materials and methods

Generation of iPSCs

Fibroblasts from an aged C57BL/6J mouse were reprogrammed using the CytoTune-iPS 2.0 Sendai reprogramming kit following the instructions of the manufacturer, with minor modifications (MOI for KLF4 = 5). This line was maintained and expanded first on feeder layers (CRL-2429, ATCC), inactivated using mitomycin-C (10 μg/mL for 2 h), using 2i/LIF medium with gelatin as matrix. The composition of the 2i/LIF medium is: neurobasal medium and DMEM/F-12 Glutamax (1:1), supplemented with 2% B27, 1% N2, 385 nM β-mercaptoethanol, 0.5% L-Glutamine, 1% penicillin/streptomycin, 1 μM PD0325901, 3 μM CT99021, 10^3 U/mL LIF. The line was afterwards adapted to feeder-free conditions, using ES-Cult™ Maintenance Medium (StemCell Technologies) and gelatin. Cells were passaged when reaching a 80–90% confluence using Accumax® (Millipore) without rock inhibitor, approximately once a week and with a 1:5 ratio of split. The incubator conditions used are 37 °C and 5% CO_2.

Phosphatase alkaline analysis

The iPSC line IISHDOi005-A was seeded on a feeder layer plate. After one week, direct phosphatase alkaline activity was determined using the phosphatase alkaline blue membrane substrate solution kit (Sigma, AB0300), (Table 1).

qPCR analysis

Total mRNA was isolated using TRI Reagent® and 1 μg was used to synthesize cDNA using the Thermo Scientific RevertAid RT Kit. The qPCR was carried out with PowerSYBR®Green PCR Master Mix (Applied Biosystems), and analysed using an Applied Biosystems™ 7500 Fast Real-Time PCR System. One μl of the reaction was used to quantify by qPCR the expression of the endogenous pluripotency associated genes *Oct4*, *Sox2* and *Nanog* (Table 1). Primers are listed in Table 2. All the expression values were normalized to the *Gapdh* gene. Plots are representative of at least three independent experiments.

Karyotype analysis

Karyotype analyses were carried out using cells with more than twenty culture passages. Briefly, cells were treated with 10 μg/mL of Colcemid (Gibco) for 90 min at 37 °C, trypsinized, treated with hypotonic solution KCl 0.075 M, and fixed with Carnoy's fixative. Cells were then dropped on a microscope glass slide and dried. Metaphase cells were G banded using Wright staining. At least 20 metaphases were karyotyped.

Immunofluorescence analysis

Cells were grown on 0.1% gelatin-coated 35 mm culture plates (81,156, Ibidi), fixed with 4% paraformaldehyde for 30 min at RT and permeabilized using TBS + (0.1% Triton X-100 in Tris-buffered saline, TBS) for 45 min. Then the cells were incubated in TBS + + (3% donkey serum, 0.3% Triton X-100 in TBS) for two hours at RT. Primary antibodies were applied overnight at 4 °C. Secondary antibodies, for two hours at RT. Nuclei were stained with DAPI (Sigma, 28718-90-3). The antibodies were diluted in TBS buffer with 3% donkey serum and 0.3% triton and are listed in Table 2.

In vitro differentiation assay

The *in vitro* pluripotency capacity of the line IISHDOi005-A was tested by spontaneous embryoid body differentiation. The protocol used has been described in detail by Galera et al., 2016, with minor modifications, using ES-Cult™ Maintenance Medium without LIF.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis: alkaline phosphatase activity	Positive	Fig. 1 panel B
	Qualitative analysis: immunocytochemistry	Positive for the pluripotency markers: SSEA1, Nanog, Oct4 and Sox2	Fig. 1 panel D
	Quantitative analysis: gene expression (qPCR)	Positive for the pluripotency markers: *Oct4*, *Sox2* and *Nanog*	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	40, XX Resolution 450–500	Fig. 1 panel F
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	8 loci, all matched	Available with the author
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: negative	Fig. 1 panel C
Differentiation potential	Embryoid body formation and directed differentiation	Positive for: smooth muscle actin (Sma), β-tubulin (Tuj1) and hepatocyte nuclear factor 4 alpha (Hnf4α)	Fig. 1 panel H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse anti-SSEA1	1:100	Abcam Cat# ab16285, RRID: AB_870663
	Rabbit anti-Nanog	1:150	Abcam Cat# ab80892, RRID: AB_2150114
	Mouse anti-Oct4	1:100	Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051
	Rabbit anti-Sox2	1:100	Thermo Fisher Scientific Cat# PA1-16968, RRID: AB_2195781
Differentiation markers	Mouse anti-β tubulin isotype III	1:400	Sigma-Aldrich Cat# T8660, RRID: AB_477590
	Mouse anti-Hnf4α	1:250	Thermo-Fisher Cat# K9218, RRID: AB_2633309
	Mouse anti-Sma	1:400	Sigma-Aldrich Cat# A2547, RRID: AB_476701
Secondary antibodies	Goat anti-mouse IgG (H + L), Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-11029, RRID: AB_2534088
	Goat anti-rabbit IgG (H + L), Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-11034, RRID: AB_2576217

Primers			
	Target	Expected size (bp)	Forward/Reverse primer (5′-3′)
Pluripotency markers (qPCR)	*Endo-Oct4*	367	GCCAGACCACCATCTGTC/CCCTGTAGCCTCATACTCT
	Endo-Sox2	290	GAGCTAGACTCCGGGCGATG/CTTAAACAAGACCACGAAAACGG
	Nanog	219	GTCTGATTCAGAAGGGCTC/CTGGACCGCTCAGTCCTG
House-Keeping Genes (qPCR)	*Gapdh*	122	AGGTCGGTGTGAACGGATTTG/TGTAGACCATGTAGTTGAGGTCA
Virus silencing	SeV	181	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS	528	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG
	Klf4	410	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA
	c-Myc	532	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG
STR analysis	18–3	149	[6FAM]TCTTTCTCCTTTTGTGTCATGC/GTTTCTTGCTAAATAACTAAGCAAGTGAACAGA
	4–2	300	[6FAM]AAGCTTCTCTGGCCATTTGA/GTTCATAAACTTCAAGCAATGACA
	6–7	420	[6FAM]AGTCCACCCAGTGCATTCTC/GTTTCTTCATGTGGCTGGTATGCTGTT
	9–2	400	[6FAM]GGATTGCCAAGAATTTGAGG/GTTTCTTTCCTGAGTTGTGGACAGGGTTA
	15–3	300	[6FAM]TCTGGGCGTGTCTGTCATAA/GTTTCTTTTCTCAGGGAGGAGTGTGCT
	6–4	295	[6FAM]TTTGCAACAGCTCAGTTTCC/GTTTCTTAATCGCTGGCAGATCTTAGG
	12–1	220	[6FAM]CAAAATTGTCATTGAACACATGTAA/GTTTCTTTCAATGGTCAAGAAATACTGAAGTACAA
	5–5	280	[6FAM]CGTTTTACCTGGCTGACACA/GTTTCTTGGTTTAAAACTCAATACCAAACAA
Mycoplasma detection	*Gpo-3/Mgso*	300	GGGAGCAAACAGGATTAGATACCCT/TGCACCATCTGTCACTCTGTTAACCTC

Mycoplasma detection

Mycoplasma detection was performed by PCR analysis using 1 mL of the cell culture supernatant (3 days culture, 90% confluence). After initial denaturation at 95 °C for 2 min, 35 cycles of amplification at 95 °C for 30 s, at 55 °C for 30 s, and at 72 °C for 1 min, and a final extension at 72 °C of 3 min were performed in an Applied Biosystems Veriti Thermal Cycler (Applied Biosystems). Primers used are specified in Table 2. The 300 bp band represents a mycoplasma-positive sample available in our laboratory (positive control, C+).

DNA fingerprinting analysis

For DNA fingerprinting analysis eight different markers (Almeida et al., 2014) have been amplified by PCR and analysed by ABI PRISM 3100 Genetic analyzer and Peak Scanner v3.5 (Applied Biosystems), (Table 2).

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